CLAIMS:

1. A method of producing a first layer electrode membrane comprising:-

(1) Forming a solution containing Linker Lipid A, the disulfide of mercaptoacetic acid (MAAD) or similar molecule, linker Gramicidin B, membrane spanning lipid C (MSL-C) and membrane spanning lipid D (MSL-D) or other suitable linker molecules and other ion channel or ionophore combinations;

(2) Contacting an electrode containing a clean gold surface with the solution, the disulfide containing components in the solution thus adsorbing onto the gold surface of the electrode;

(3) Rinsing the electrode with a suitable organic solvent; and

(4) Removing the excess organic solvent used for rinsing.

2. A method according to claim 1, wherein the solution contains the disulfide of mercaptoacetic acid (MAAD)or 2-mercaptoethanol (EDS).

3. A method according to claim 2, wherein the ratio of Linker Lipid A to the disulfide of mercaptoacetic acid (MAAD) or 2-mercaptoethanol (EDS) is 2:1.

4. A method according to claim 2 or 3, wherein the ratio of (Linker Lipid A + MAAD or EDS) to MSL-D is in the range of 10:1 to 100:1.

5. A method according to any one of claims 2 to 4, wherein the ratio of (Linker Lipid A + MAAD or EDS) to MSL-C is between 20,000:1 and 100:1.

6. A method according to any one of claims 2 to 5, wherein the ratio of (Linker Lipid A + MAAD or EDS) to MSL-C is 20,000:1.

7. A method according to any one of claims 2 to 6, wherein the solution contains linker Gramicidin B rather than another suitable linker molecule/ion channel or other combination.

8. A method according to claim 7, wherein the ratio of (Linker Lipid A + MAAD or EDS) to linker Gramicidin B is 10,000:1.

~~9.~~ A method according to claim ~~7 or 8,~~ wherein the ratio of (Linker Lipid A + MAAD or EDS) to linker Gramicidin B is between 20,000:1 and 100,000:1.

~~10.~~ A method according to ~~any one of the preceding~~ claims wherein the gold electrode consists of a freshly evaporated or sputtered gold electrode.

~~11.~~ A method according to claim ~~10~~, wherein the gold electrode surface is freshly cleaned using a plasma etching process or an ion beam milling process.

~~12.~~ A method according to ~~any one of the preceding~~ claims wherein the solvent for the adsorbing solution (step (1)) and for the rinsing step (4) is ethanol.

13. A method of producing a monolayer electrode membrane comprising:-

(1) Forming a solution containing the disulfide of mercaptoacetic acid (MAAD) or similar molecule, membrane spanning lipid C (MSL-C) and/or membrane spanning lipid D (MSL-D) and, optionally, Linker Lipid A, linker Gramicidin B or other suitable linker molecules and other ion channel combinations;

(2) Contacting an electrode containing a clean gold surface with the solution, the disulfide containing components in the solution thus adsorbing onto the gold surface of the electrode;

(3) Rinsing the electrode with a suitable organic solvent; and

(4) Removing the excess organic solvent used for rinsing,

wherein the solution in step (1) contains more than a molar % of 50% of a membrane spanning lipid.

14. A method according to claim 13, wherein the solution in step (1) contains more than a molar % of 70% of a membrane spanning lipid, 29% MAAD or 2-mercaptoethanol (EDS) and 1% other membrane spanning lipids.

15. A method according to claim 13 or 14, wherein the gold electrode consists of a freshly evaporated or sputtered gold electrode.

16. A method according to claim 15 wherein the gold electrode surface is freshly cleaned using a plasma etching process or an ion beam milling process.

17. A method according to any one of claims 13 to 16, wherein the solvent for the adsorbing solution (step (1)) and for the rinsing step (4) is ethanol.

18. A method according to any one of claims 13 to 17, wherein MAAD, or similar spacer molecule, such as EDS is covalently linked to the membrane spanning lipids C or D.

19. A method according to any one of claims 13 to 18, wherein MSL-C or D is covalently linked to DPEPC, GDPE, triphytanyl or similar molecule.

20. A method according to any one of claims 13 to 19, wherein the solution contains the disulfide of mercaptoacetic acid (MAAD) or 2-mercaptoethanol (EDS).

21. A method according to claim 24, wherein MSL-C or D is covalently linked to MAAD or EDS and DPEPC, GDPE, triphytanyl or similar molecule.

22. A method of producing a second layer electrode membrane combination comprising:-

(1) Adding a solution of lipid and biotinylated gramicidin E dispersed in a suitable solvent onto the electrode surface containing a first layer produced by a method according to any one of claims 1 to 12;

(2) Rinsing the electrode surface with an aqueous solution;

(3) Adding a solution of streptavidin, avidin, neutravidin, avidin or streptavidin derivative;

(4) Rinsing the electrode with an aqueous solution in order to remove excess streptavidin, avidin, neutravidin, or other avidin or streptavidin derivative;

(5) Adding a solution of a biotinylated binding partner molecule; and

(6) Rinsing the coated electrode with an aqueous solution.

23. A method according to claim 22, wherein the lipid used in step (1) is a mixture of diphytanyl phospatidyl choline (DPEPC) and glyceryl diphytanyl ether (GDPE).

24. A method according to claim 22 ~~or 23,~~ wherein the DPEPC and GDPE is in a 7:3 ratio.

25. A method according to claim 22, wherein the lipid used in step (1) is a triphytanyl phosphoryl choline as shown in Figure (6).

26. A method according to claim 22, wherein 0-50% cholesterol is incorporated into the lipids used in step (1).

27. A method according to claim 22, wherein 0-20% cholesterol is incorporated into the lipids used in step (1)

28. A method according to any one of claims 22 ~~to 27,~~ wherein the ratio of lipid to biotinylated gramicidin E is between 10,000:1 and 1,000,000:1.

29. A method according to claim 28, wherein the ratio of lipid to biotinylated gramicidin E is 100,000:1.

30. A method according to any one of claims 22 ~~to 29~~, wherein the biotin is attached to the gramicidin via the ethanolamine end using a linker arm that is made up of between 1-8 aminocaproyl groups.

31. A method according to any one of claims 22 ~~to 30,~~ wherein two biotins are attached to the gramicidin at the ethanolamine end such that the biotins are able to bind simultaneously into the adjacent binding sites of a streptavidin, avidin or a similar biotin-binding protein.

32. A method according to any one of claims 22 ~~to 30~~, wherein two biotins are attached to the gramicidin at the ethanolamine end such that the biotins are able to bind simultaneously into two separate streptavidin, avidin or a similar biotin-binding protein molecules.

33. A method according to claim 31, wherein the two biotins are attached to the gramicidin via the ethanolamine end such that each biotin is attached to 2 to 4 linearly joined aminocaproyl groups that are attached to a lysine groups as shown in Figure (5).

34. A method according to claim 31, wherein the biotins are attached to the gramicidin via the ethanolamine end such that each biotin is attached to 2 to 20 linearly joined aminocaproyl groups that are attached to a lysine groups.

35. A method according to claim 30, wherein the biotins are attached to the gramicidin via the ethanolamine end such that each biotin is attached to 2 to 20 aminocaproyl groups arranged as a branched structure.

36. A method according to any one of claims 22 ~~to 33~~, wherein the amount of streptavidin, avidin or other similar biotin-binding protein that is added in step (3) is sufficient to cause a prozone effect, allowing most of the available biotinylated species in the membrane to have one streptavidin or related molecule bound to prevent cross-linking between gramicidin channels and MSL until a sample containing analyte is added to the sensor.

37. A method according to any one of claims 22 ~~to 36~~, wherein prior to the addition of streptavidin, avidin or similar biotin-binding protein, the lipid membrane electrode assembly is cooled.

38. A method according to claim 37, wherein the lipid membrane electrode assembly is cooled to between 0° and 50°C.

39. A method according to claim 38, wherein the lipid membrane electrode assembly is cooled to between 0° and 5°C.

40. A method according to any one of claims 37 ~~to 39~~, wherein the subsequent rinsing and addition of the biotinylated binding partner molecule is carried out at 0° to 50°C.

41. A method according to claim 40, wherein the subsequent rinsing and addition of the biotinylated binding partner molecule is carried out at 0° to 5°C.

42. A method according to claims 40 ~~or 41~~, wherein the binding partner molecule is a biotinylated antibody or biotinylated antibody fragment.

43. A method according to any one of claims 37 ~~to 39~~, wherein the binding partner molecule is a Fab' fragment that is biotinylated via the free Fab' thiol group.

44. A method according to claim 40, wherein the linker between the Fab' and biotins is between 10-80 angstroms in length.

45. A method according to claim 42 ~~or 43~~, wherein the linker between the Fab' and biotins consists of 1-8 aminocaproyl groups.

46. A method according to claim any one of claims 42 ~~to 45~~, wherein the group containing two biotins is attached to the antibody or antibody fragment such that the two biotins are able to simultaneously complex one streptavidin, avidin or other similar biotin-binding proteins.

47. A method according to claim any one of claims 42 ~~to 45~~, wherein the group containing two biotins is attached to the antibody or antibody fragment such that the two biotins are able to complex simultaneously two streptavidin, avidin or other similar biotin-binding proteins.

48. A method according to any one of claims 22 ~~to 41~~, wherein steps (3) to (5) are substituted with:

(3) Adding a solution containing a conjugate between streptavidin, avidin, neutravidin or other avidin or streptavidin derivative and a molecule which is a member of a binding pair.

49. A method according to claim 48 wherein the binding partner molecule is an antibody or an antibody fragment such as a Fab, Fab' or Fabv fragment.

50. A method according to claim 48 ~~or 49~~, wherein the binding pairs are selected from: naturally occurring binding proteins and cellular receptors/analytes, enzymes or enzyme analogues/substrates, lectins/carbohydrates, complementary nucleic acid sequences, Anti-FC, Protein A or Protein G/antibody.

51. A method of producing a second layer electrode membrane combination comprising:-

(1) Adding a solution of lipid dispersed in a suitable solvent onto the electrode surface containing a first layer produced according to any one of claims 1 to 12;

(2) Rinsing the electrode surface with an aqueous solution;

(3) adding an aqueous solution containing ionophore co-dispersed with detergent or solubilised by coupling to a high molecular weight species;

(4) Rinsing the electrode with an aqueous solution; and

(5) Adding the receptor using either streptavidin, avidin or other similar biotin-binding protein followed by addition of a biotinylated antibody or antibody fragment or adding a streptavidin, avidin or other similar biotin-binding protein conjugated to an antibody or antibody fragment.

52. A method according to claim 51, wherein the lipid used in step (1) is a mixture of diphytanyl phosphatidyl choline (DPEPC) and glyceryl diphytanyl ether (GDPE).

53. A method according to claim 52, wherein the DPEPC and GDPE is in a 7:3 ratio.

54. A method according to claim 51, wherein the lipid used in step (1) is a triphytanyl phosphoryl choline as shown in Figure (6).

55. A method according to any one of claims 51 ~~to 54,~~ wherein the membrane produced also contains 0-50% cholesterol.

56. A method according to any one of claims 51 ~~to 54~~, wherein the membrane produced also contains 0-20% cholesterol.

57. A method according to any one of claims 51 ~~to 56~~, wherein the aqueous solution used in step (3) contains gramicidin or a gramicidin derivative that is added to an aqueous solution of a detergent such that the detergent is present in excess relative to the gramicidin but wherein the total concentration of the detergent is below the critical micelle concentration (CMC).

58. A method according to claim 57, wherein the gramicidin/detergent solution is sonicated using an ultrasonic bath or horn for 5-20 minutes.

59. A method according to claim 57 ~~or 58~~, wherein the detergent is selected from sodium dodecylsulfate, octylglucoside, tween, and other ionic or non-ionic detergents.

60. A method according to claim 59, wherein the detergent is sodium dodecylsulfate.

61. A method according to claim 60, wherein the concentration of the sodium dodecylsulfate is less than 0.00001M and the concentration of gramicidin is 10 times less than the sodium dodecylsulfate concentration.

62. A first layer membrane electrode combination comprising an electrode and a first layer membrane comprising a closely packed array of amphiphilic molecules and a plurality of ionophores, the first layer membrane being connected to the electrode by means of a linker group, said first layer membrane being stored in the presence of a solvent.

63. An electrode combination according to claim 62, wherein the solvent in which the electrodes are stored is an organic solvent or an aqueous solvent.

64. An electrode combination according to claim 63, wherein the solvent in which the electrodes are stored is selected from ethanol, glycerol, ethylene glycol and alcohols or diols containing between 3 to 12 carbon atoms.

65. An electrode combination according to claim 63, wherein the solvent in which the electrodes are stored is a hydrocarbon with between 8 to 20 carbon atoms.

66. An electrode combination according to claim 63, wherein the solvent is an aqueous solution containing a detergent.

67. An electrode combination according to claim 62 ~~or 63~~, wherein the solvent in which the electrodes are stored is a compound that is able to coat the electrodes such that oxidation of the electrode surface is minimised.

68. An electrode combination according to claim 67, wherein the solvent can be applied as a thin film.

69. A lipid membrane based biosensor comprising a lipid membrane incorporating ionophores, the conductivity of the membrane being dependent on the presence or absence of an analyte, wherein the aqueous bathing solution in which the biosensor normally resides, is removed in a manner such that on drying of said lipid membrane biosensor, the lipid membrane and the receptor molecules retain their function, structure and activity, when rehydrated.

70. A biosensor according to claim 69, such that during the drying process the biosensor membrane does not have contact with the air-water interface.

71. A biosensor according to claim 69, such that during the rehydration process the biosensor membrane does not have contact with the air-water interface.

72. A biosensor according to any one of claims 69 ~~to 71~~, wherein the aqueous bathing solution is removed by a method of drying selected from lyophilysation, evaporation, and evaporation over controlled humidity.

73. A biosensor according to any one of claims 69 ~~to 72~~, wherein the aqueous bathing solution is replaced with a water replacing substance selected from protein, low molecular weight diols or triols, polyethylene glycol, low molecular weight sugars, polymeric peptides, polyelectrolyte and combinations thereof.

74. A biosensor according to claim 73, wherein the water replacing substance is selected from bovine serum albumin, serum, fish gelatin, non-fat dry milk powder, casein, glycerol, ethylene glycol, diethylene glycol, polyethylene glycol, trehalose, xylose, glucose, sucrose, dextrose, dextran or ficoll.

75. A biosensor according to ~~claim 74~~, wherein the water replacing substance is selected from glycerol, sucrose, dextran or trehalose.

76. A biosensor according to claim 73, wherein the class of molecules are chosen such that they have the additional advantage of providing a spreading layer for the sample.

77. A biosensor according to claim 73, wherein the class of molecules are chosen such that they have the additional advantage of providing filter against specific cells, bacteria, viruses and classes of molecules, such as large moleculer weight proteins.

78. A biosensor according to claim 73, wherein the class of molecules are chosen such that they have the additional advantage of providing a reservoir for specific displacement reagents, which are required to compete off small analytes bound to proteins in serum or blood.

79. A biosensor according to claim 73, where any of water replacing agents may be bound covalently to specific membrane components.

80. A biosensor according to claim 73, where any of water replacing agents may be bound covalently to membrane spanning lipids.

81. A method according to any one of claims 13 ~~to 21~~, wherein valinomycin is covalently linked to the MSL-C or MSL-D via a linker of appropriate length to permit the valinomycin to diffuse from one side of the membrane to another.

82. A method according to claim 1 ~~or 2~~, wherein the ratio of Lipid Linker A to the disulfide of mercaptoacetic acid (MAAD) or 2-mercaptoethanol (EDS) is in the range of 5:1 to 1:2.